Tavora *et al.*
Surgical and Experimental Pathology (2023) 6:13
https://doi.org/10.1186/s42047-023-00138-w

Surgical and Experimental Pathology

REVIEW

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Guidelines for molecular testing in non-small cell lung cancer – recommendations from the Brazilian Society of Pathology

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Abstract

The Brazilian Society of Pathology Guidelines project aims to provide recommendations for clinicians and pathologists based on the best available scientific evidence adapted from the International Guidelines, with emphasis in the practice of Brazilian pathologists. It reviews currently available and emerging molecular tests. In this paper, a combined effort from members of the Brazilian Society of Pathology describes the essential pre-analytical issues, the required clinical information to allow proper molecular testing interpretation, and the important role of pathologists in multidisciplinary tumor boards.

Keywords Lung cancer, Non-small cell lung cancer, EGFR, ALK, Tumor board

Introduction

There has been a need in the past 10 years to standardize sample preparation, analysis, and reporting of lung cancer to maximize molecular testing possibilitiesi (Penault-Llorca et al. 2022a). The Brazilian Society of Pathologists (SBP) has therefore formed a working group to analyze literature and recommendations from international societies, adapt them to Brazilian pathology practices, and provide guidance to pathologists. This article is a product of this effort, supported by the Brazilian Society of Clinical Oncology (SBOC), the Brazilian Society of Thoracic Surgeons (SBCT), and the Brazilian Group of Thoracic Oncology (GBOT). The ultimate goal is to enhance communication with the multidisciplinary team (MDT) for optimal management of lung tumors. This article does not cover the classification of lung tumors, the histologic subtypes nor the current WHO nomenclature.

As an increasing number of biomarker-guided therapies for lung cancer gain approval, molecular testing algorithms continue to evolve. Numerous potential algorithms exist to fulfill the necessary objectives (Penault-Llorca et al. 2022a, 2022b; Dietel et al. 2016; Lindeman et al. 2018a, 2018b; John et al. 2021). While this article presents some possibilities, with emphasis in the practice of Brazilian pathologists, it also underscores the underlying principles on which alternative versions can be developed to meet specific local requirements and leaves the practice open to new developments and discoveries.

In a recent article analyzing practices in Brazil, medical oncologists reported several specific challenges

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associated with ordering comprehensive genomic profiling. These challenges include a significantly long turnaround time for sample analysis and result delivery, lack of access to treatments or clinical trials targeting the genomic alterations identified through sequencing tests, high cost and lack of reimbursement and challenges related to the sample itself, such as poor sample quality and bureaucratic hurdles involved in sending the sample for analysis (Fares et al. 2022).

In the context of lung cancer, most molecular testing is performed on biopsy specimens since 60% to 70% of patients receive a clinical diagnosis at an advanced stage (IIIB-IV). Regardless of the extent of testing, three fundamental principles guide this process:

1. Expediency: Turnaround time is crucial as patients with advanced lung cancer may deteriorate rapidly. Prompt delivery of results is essential.
2. Accuracy: Confirming the cancer diagnosis and ensuring precise molecular profiling require accuracy. Reliable and precise testing methods are vital for accurate treatment decisions.
3. Comprehensive Testing: Thorough gene profiling is vital to cover all relevant targets with effective drugs. This approach ensures no actionable targets are missed and identifies appropriate treatment options.

By adhering to these principles, pathologists and molecular biologists can optimize patient care in lung cancer molecular testing, facilitating timely and accurate diagnosis and personalized treatment strategies. A template of the reporting guide will be available online and a Portuguese edition will beavailable on the website of the Brazilian Society of Pathology: https://www.sbp.org.br/.

Methods

During 2022, the Brazilian Society of Pathologists gathered a group of pathologist members with interest in pulmonary and molecular pathology in an online and physical meeting at its headquarters to draft the proposal of recommendations for processing, diagnosis and biomarker testing in lung tumor samples. In subsequent meetings, renowned Brazilian oncologists and thoracic surgeons were invited to comment on the proposal. The working group had the task of writing this manuscript in English, to be published as reference, and write an online guideline in Portuguese, that will be available at www.sbp.org.br.

Recent guidelines from the College of American Pathologists (CAP), Association for Molecular Pathology (AMP), and the International Association for the Study of Lung Cancer (IALSC), were used as starting references, as well as the most current National Comprehensive Cancer Network (NCCN) guideline (Passaro et al. 2022; Mosele et al. 2020; Kalemkerian et al. 2018; Smeltzer et al. 2020; Sholl et al. 2023). A comprehensive literature review from pubmed/Medline was performed by the authors.

Tissue collection and management

Pathological diagnosis in lung cancer extends beyond histological classification, encompassing molecular classification and predictive biomarker reporting. Various methods exist for obtaining samples from malignant lung neoplasms (Fig. 1). Small diagnostic samples (biopsies or cytology) are increasingly common, surpassing materials from complete surgical resection (Bubendorf et al. 2017; Gill et al. 2018). For the 70% of lung cancer patients with unresectable advanced-stage disease, diagnosis and biomarker procurement primarily rely on small biopsies and cytology samples (Hess et al. 2022).

The rise in small samples is attributed to advancements in imaging and intervention techniques, screening programs (detecting lesions earlier), less invasive and safer surgical approaches, and the higher frequency of initial diagnoses of inoperable advanced-stage disease (mainly using small samples). Consequently, managing these small samples from primary or metastatic tumors becomes more challenging, with few viable neoplastic cells and potential undersampling of heterogeneous tumor areas (both histologically and biologically).

Clinical-radiological correlation is essential for high-quality management of these samples, guiding histopathological diagnosis and, most importantly, the composition of the immunohistochemical panel. The following characteristics are crucial:

1. Lesion location and radiological characteristics of the lesion.
2. Patient's medical history, particularly the presence of extrapulmonary tumors.
3. Risk factors such as smoking and family history of neoplasia.
4. Specification of the method used for sample acquisition.
5. Exact time of the procedure and the time the tissue was placed in buffered 10% formalin.

If this information is not provided in the medical request, it is recommended for the pathologist to contact the attending physicians to gather these details, as they directly influence the diagnostic approach to these materials. Institutions that invest in multidisciplinary meetings achieve faster, more effective, and efficient pathological and molecular diagnoses (see MDT section

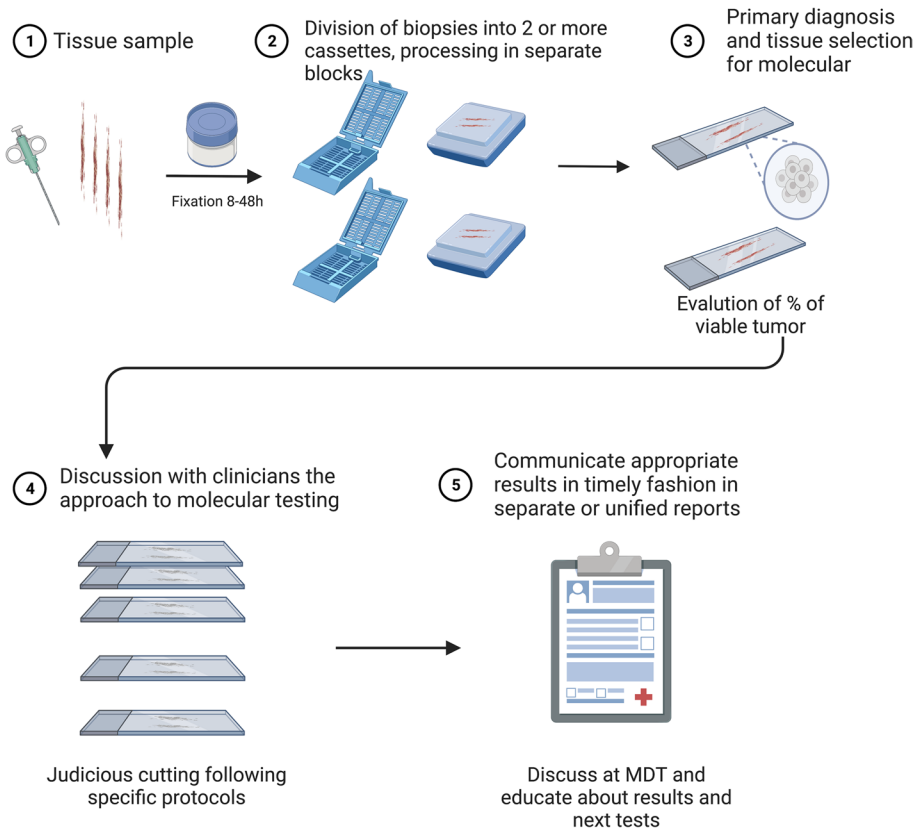

Fig. 1 The biopsy journey. The pathologist is responsible to design a flow that allows rapid turnaround time at the same time as maximizing tissue availability for molecular studies. Created with BioRender.com

below). The success of pathological and molecular diagnoses is closely related to pre-analytical factors (such as fixative, appropriate volume, and fixation times).

Fixation and processing

SBP recommends that clinicians (such as interventional radiologists, pulmonologists, and thoracic surgeons) are educated by pathologists to fix surgical samples—such as biopsies and FNA—in buffered 10% formalin immediately upon acquisition. The sample must then be sent promptly to the laboratory where a pathologist's assistant should fix it for at least 6 h but no longer than 48 h (preferably around 24 h) so that protein and DNA fragmentation are minimized. To guarantee optimal preservation of tissue structure for future analyses like H&E staining, IHC, FISH or DNA/RNA sequencing, fixation parameters which include time allowed for fixing and volume used need to be optimized ensuring maximum chances of excellent downstream analysis opportunities (Hess et al. 2022). Tumor boards are a great moment to show current data, and to reinforce the need to follow pre-analytical standard procedures.

Tissue sectioning procedure should also be designed to minimize potential tissue waste. Laboratories should develop their own optimal protocol based on available capacity. However, a range of strategies including superficial sectioning of a single slide to pre-sectioning of multiple unstained slides with pre- and post-assessment of hematoxylin and eosin (H&E) concentrations required for DNA and RNA extraction. Precautions should be taken at all points of processing and cutting to avoid cross-contamination. This may require the use of a special microtome and a new, unused blade each time a section is cut for DNA/RNA extraction.

Role of the pathologist in assessing tissue quality

Separate core needles or bronchial biopsies should ideally be distributed into separate blocks, completely in one block, so that one block can be used for diagnostic staining and the remaining block can be saved for molecular testing. This approach requires that all fragments contain tumor, and this should be evaluated by the surgical pathologists, with indication of the best block for molecular analyses in the report as a comment.

Pathologist should be aware and guarantee that molecular analysis is performed on slides that accurately reflect the diagnosis of the pathology report, and they should select and mark slides without significant amounts of necrosis, inflammation or fibrosis, if possible. n patients with multiple samples, the most recent tissue should be used (Russo et al. 2022).

The percentage of tumor cells (to the total number of nucleated cells) should be annotated and ideally included in a comment in the pathology report. This facilitates the process if the case is sent out to molecular analyses in a different laboratory. In the latter, ideally an additional H&E should be cut and stained as the last slide of the process, to check for accurate percentage (Gullo et al. 2020; Pei et al. 2019; Cree et al. 2014).

Macrodissection is an important technique for enriching tumor cells, highlighting the critical role of pathologists. This technique improves the accuracy of direct or next-generation sequencing molecular analysis (Gullo et al. 2020). In some cases, the amount (volume or area) of tumor tissue is not always sufficient for biomarker testing (Penault-Llorca et al. 2022c). The minimum amount of tumor DNA/RNA and of malignant cells required varies and depends on the analytical sensitivity of the particular molecular test (Dufraing et al. 2019).

Processing of cytology and rapid on-site evaluation (ROSE)

ROSE is recommended by the NCCN Guidelines ensure transbronchial needle aspirates (TBNAs) or EBUS samples are adequate for diagnosis and posterior molecular testing. It requires the presence of the pathologists in the operating room or bronchoscopy suite, to confirm the presence of available material (Fassina et al. 2010; Jain et al. 2017). ROSE may also help to ensure sample adequacy and sufficient yield for cancer subtyping and molecular testing (Sung et al. 2020). ROSE helps achieve relatively high sampling success rates and reduces the number of aspirations required. However, ROSE can be costly (often with limited health insurance reimbursement), and time consuming (Fassina et al. 2010; Jain et al. 2017; Fox et al. 2021; Botticella et al. 2021).

Tumor diagnosis and subtyping

Although not the primary focus of this study, tumor diagnosis and subtyping are crucial responsibilities of surgical pathologists. Additionally, accurate interpretation of the tumor facilitates the preservation of tissue for further molecular investigations. Establishing a malignancy diagnosis and distinguishing between carcinoma and other tumor types, including non-small cell cancer, typically requires only an H&E stain. However, additional questions may arise regarding the possibility of neuroendocrine differentiation and whether the tumor is a primary lung cancer. The 2021 WHO Thoracic Classification of Tumours provides well-established recommendations on diagnostic approaches in such cases. (Classification and of Tumours Editorial Board, editor. Thoracic Tumours: WHO Classification of Tumours. 2021).

In this context, considering the patient's overall clinical status and knowledge of imaging findings can help prevent unnecessary immunohistochemistry and conserve valuable tissue. With appropriate clinical correlation, it has been estimated that a more specific diagnosis than non-small cell carcinoma can be achieved with H&E staining alone in approximately 70% of transthoracic biopsies (Loo et al. 2010; Oliveira et al. 2019). Immunohistochemistry should be reserved for the remaining cases or rare instances where clinical and morphological evidence suggests metastasis from another primary source (Bubendorf et al. 2017; Loo et al. 2010; Edwards 2000; Thunnissen 2018).

The current guideline suggests providing an accurate histological/cytological diagnosis (specific subtype of non-small cell carcinomas) and determine the origin (primary or metastatic) in adenocarcinomas. In addition to routine traditional techniques, auxiliary techniques (such as immunohistochemistry and special stains) can be used while preserving the sample as much as possible for molecular testing. Tissue sampling poses a significant challenge in the era of personalized molecular therapies and immunotherapy. Histological and biological heterogeneities are well-known phenomena that can significantly affect the ability to detect and predict the response to therapy targeting specific molecular targets. The smaller the sample, the lower the probability of representing tumor heterogeneity. Therefore, a multidisciplinary strategy is essential to obtain adequate biopsies not only for diagnosis but also for molecular and biomarker testing. A limited panel of immunohistochemical markers can reliably distinguish histological types in most non-small cell lung carcinomas, allowing tissue preservation for molecular testing. (Figs. 1, 2, 3, and 4).

When necessary, a small panel consisting of TTF-1 and p40 or p63 antibodies is generally sufficient for most cases. Work-up with large panels using more than 2–4 antibodies is strongly discouraged. Laboratories with established protocols for IHC biomarkers and/or molecular testing can take advantage of this stage to prepare additional slides for RT-PCR (commonly EGFR), PD-L1, ALK, or multiple slides for NGS testing (Figs. 2 and 3). Unfortunately, it should be noted that most referral laboratories in Brazil do not accept unstained slides for NGS testing; they only require original paraffin blocks. Therefore, in such scenarios, an additional H&E slide should

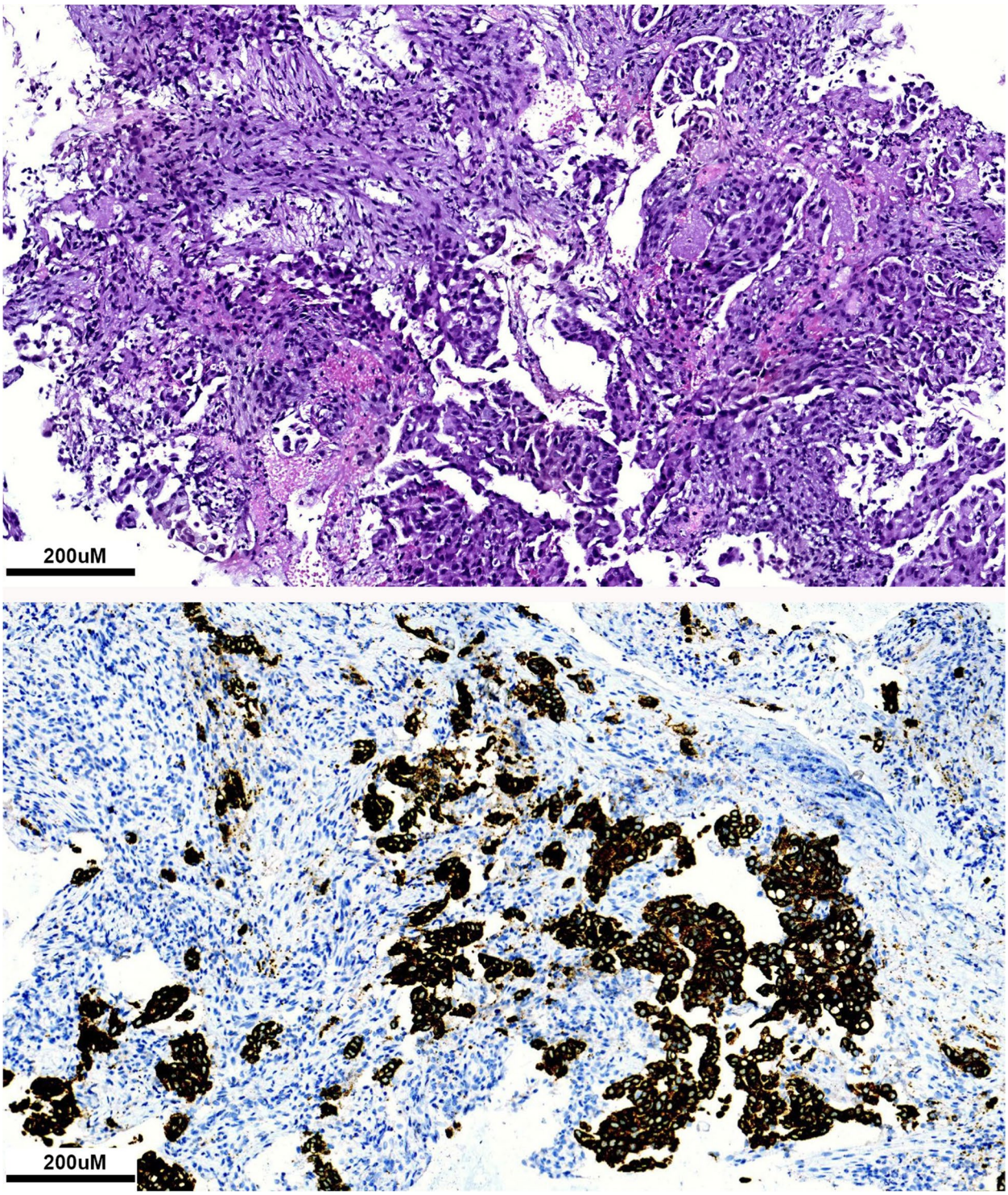

Fig. 2 ALK gene status determined by the Ventana D5F3 IHC in a primary lung adenocarcinoma, solid subtype. **A**—H&E stain and **B** IHC stain and strong immunoreactivity of ALK-D5F3 in the tumor

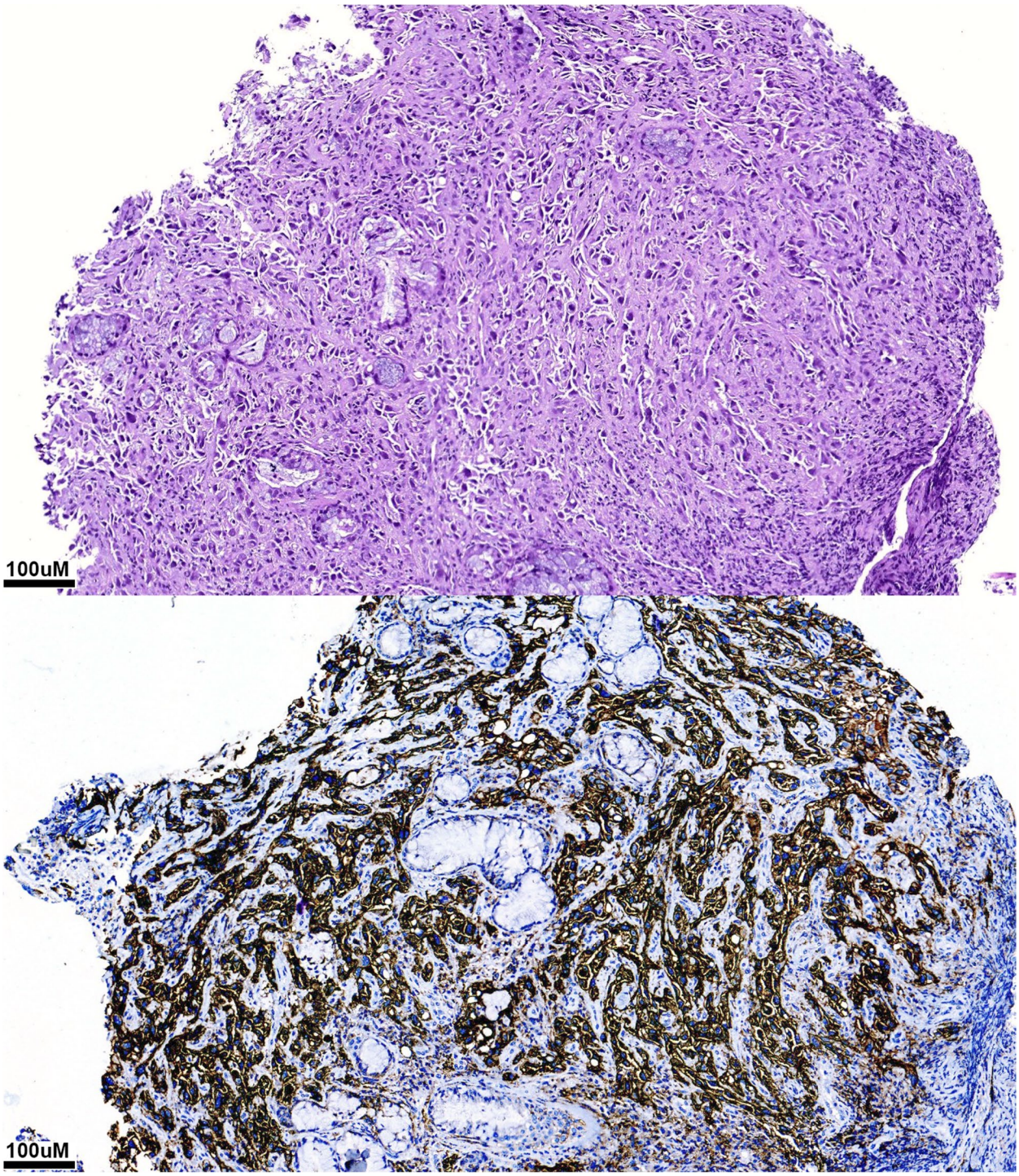

Fig. 3 **A** High-power view (100x) of transbronchial biopsy showing solid type adenocarcinoma. **B** Immunohistochemical stain (Clone 22c3) representative micrograph of a high PD-L1 tumor proportion score (TPS) ≥ 50%

be performed at the end of the cutting process to accurately estimate the percentage of tumor tissue available for molecular testing (Aggarwal et al. 2021; Yatabe et al. 2019).

PD-L1 testing

In several Brazilian labs, only IHC technique is available. Thus, PD-L1 test (and ALK) may be the only in-house companion diagnostic performed before sending the

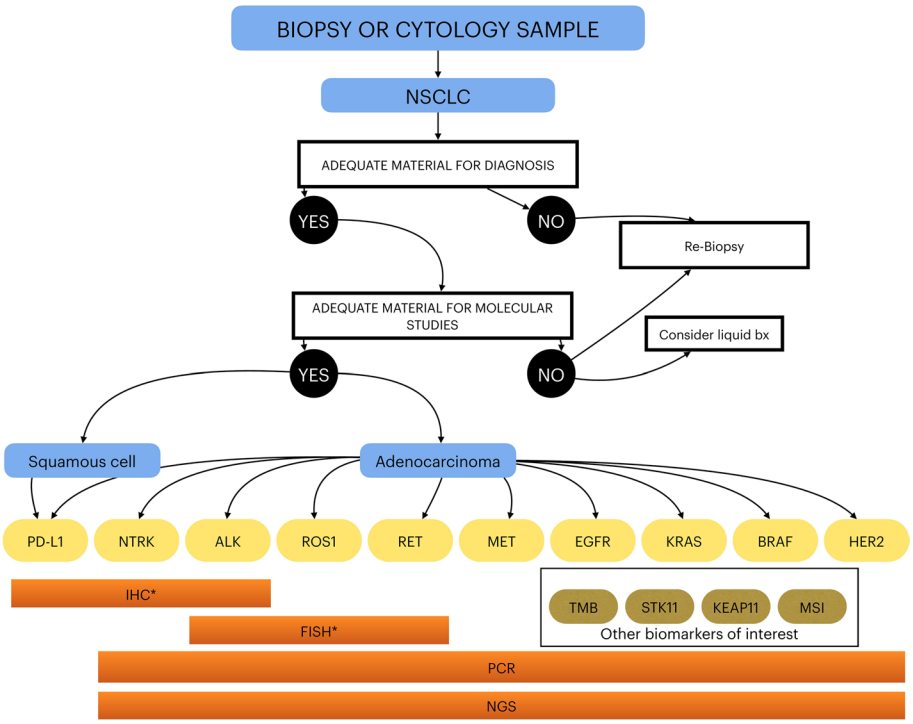

Fig. 4 Diagnostic algorithm with small samples in patients with non-small cell lung carcinoma (NSCLC). * IHC and FISH should be used in validated labs. IHC for NTRK and ROS1 can be used only as a screening tool, to be confirmed by FISH, NGS or PCR. In most cases, NGS should be the preferred method up front

case out for other molecular markers. PD-L1 is currently based on immunohistochemistry (IHC) and is the only validated predictive test. The variety of IHC tests and cutoffs that define positive results has caused confusion and prompted efforts to harmonize within the scientific community (Cheung et al. 2019). Current guidelines recommend standard preanalytical conditions for the measurement of PD-L1 biomarkers by IHC. PD-L1 expression is assessed by determining the percentage of tumor cells exhibiting partial or complete membrane staining of any intensity (TPS or TC), immune cells expressing cytoplasmic PD-L1 (IC), or both (CPS) (Tsao et al. 2018; Forde et al. 2018; Hirsch et al. 2017).

Several PD-L1 clones are available for IHC testing. The four most commly used clones in pathology laboratories are Agilent's 22C3 and 28–8 (using the Agilent Autostainer LINK 48® platform), MedImmune®/ Ventana®'s SP263, and Spring®/Bioscience®/ SP142 from Ventana® using the Agilent Autostainer LINK 48® platform. Agilent Autostainer LINK 48® platform) (Tsao et al. 2018; Hirsch et al. 2017; Vennapusa et al. 2019; Rimm et al. 2017). The performance characteristics of the 22C3 and 28–8 tests appear similar based on parallel evaluations in retrospective cohorts. SP263 and E1L3N, although used in routine practice, are not approved as adjunctive diagnostic tests, but if properly validated, may show staining patterns comparable to approved tests. However, the SP142 assay consistently shows less tumor cell staining, even though the SP142 antibody recognizes an identical or nearly identical epitope to his SP263 and E1L3N (Dodson et al. 2020; Krawczyk et al. 2017). The SP142 assay is optimized for evaluating both tumor and immune cells. However, its performance as an immune cell marker is further complicated by the lack of agreement among observers in the interpretation of immune cell expression (Krawczyk et al. 2017). Of note, TPS is the preferred method to assess PD-L1 expressoin levels for routine clinical decision making Table 1.

Regarding sample selection, if multiple tissue blocks are available for a given tumor, the most representative sample should be analyzed, like the choice for molecular studies. Since the techniques in some labs require additional cutting, PD-L1 can be performed in the same block as the diagnostic IHC. Additional blocks may be analyzed if the pathologist determines that additional testing is necessary to determine the PD-L1 status of the tumor. When analyzing additional blocks of the same sample, the results of all blocks analyzed should be combined as if they were present in a single paraffin block (Cheung et al. 2019).

Table 1 Recommendations for biopsy/cytology specimen acquisition and processing. These should be discussed in each institution and modified by the pathology according to current practices. Lindeman et al. 2018a; Roy-Chowdhuri et al. 2020; Aisner 2018

	Recommendation	Comment
Needle	• 14-20G for core needle biopsies • 20-25G for FNA • 19–20-21G for EBUS	
Number of fragments/passes	• 3 core needle biopsies minimum • EBUS – 3 to 5 passes • Multiple passes for transthoracic FNA	ROSE can be performed if the pathologist and clinical team have established protocols. If not, tissue should be maximized in cell block preparations
Time to fixation	Cold ischemia of less than 1 h	
Fixative	10% neutral buffered formalin	EDTA decalcification protocols may be used if necessary. Decalcification with acids is not recommended
Fixation	6-48h	Time of the sample in formalin should be informed in the requisition
Tissue blocks	Samples should be ideally separated in more than 1 block for processing and cutting	
Tissue selection for molecular and IHC analyses	Pathologists should choose the block with maximal amount of tumor % and register in the report	Tissue % can be enriched by macro or microdissection

It is not uncommon for the only material available to be a cell block. In such cases, PD-L1 validated against formalin-fixed paraffin-embedded (FFPE) biopsy specimens can be used, provided the cytological specimens were processed under the same required pre-analytical conditions (Rekhtman and Roy-Chowdhuri 2016; Wang et al. 2018).

Analysis and reporting

The Society highlights the important role of the pathologist in all phases of reporting the diagnosis and biomarker findings in lung cancer. Accurate and clear reporting is essential to timely decision, ideally before any treatment has been initiated. While there are many regional differences, a timeline of 10 days from initial biopsy to diagnosis and biomarker reporting is considered acceptable currently. In Brazil, in particular, since tissue blocks travel often to molecular referral outside of states, it is important that from the first diagnostic report to the complementary molecular report, information that clearly states which block, cellularity, presence of necrosis and fibrosis, need for microdissection, and sample quality that might compromise examination results. Table 2 summarizes guidelines from ESMO and CAP. These recommendations also follow the Brazilian Laboratory Quality Control—PACQ (https://pacq.sbp.org.br/).

Table 2 Recommendations around key aspects of analysis and reporting

Category	Key recommendations	Additional considerations
Diagnostic report	• Clearly state the final diagnosis and need for additional IHC • Clearly state the best block for molecular studies, and the percentage of tumor in that block, with a rough estimation of tumor volume if scant • Comment on sample quality and suitability • Follow local guidelines to indicate what tests are recommended and if referral is necessary • Testing for biomarkers mandatory for initial treatment of NSCLC must be completed within 10 working days	Results should be ideally discussed in MDT board meeting
Types of tests	• When multiple genes are going to be tested, NGS is more cost-effective • PD-L1 detection should be performed by IHC • Single-gene testing can be performed by RT-PCR • ALK IHC can be performed o assess ALK fusion status • NTRK and ROS1 IHC can be used as screening, but need confirmatory by additional methods • For RET fusions, IHC is not recommended	In advances NSCLC, combined RNA/DNA NGS with PD-L1 immunohistochemistry is probably the ideal scenario, if available and the cost is covered

Limited versus expanded panels

In the current Brazilian context, there is a lack of data supporting the use of universal NGS testing for all patients diagnosed with lung cancer. Therefore, the decision to perform molecular testing should be based on current evidence and the potential for actionable results. Pathologists play a pivotal role in multidisciplinary team (MDT) meetings by educating the team about the different types of testing available, assessing each case individually to determine the feasibility of a valid test based on factors such as tissue percentage, volume, and viability. Pathologists also provide explanations for each test result and assist in selecting the appropriate technique for each specific target. Additionally, pathologists are responsible for ensuring the utilization of the best tissue block and may recommend the need for dissection or obtaining a new sample (Fig 4).

Currently, there is sufficient evidence to support testing for PD-L1 using IHC, EGFR using RT-PCR or NGS, and ALK using IHC or within an NGS panel for patients with stage I to III NSCLC who require systemic therapy (Aggarwal et al. 2021, 2023). In early-stage patients, identifying a driver mutation can provide valuable information, as it helps identify individuals for whom immunotherapy may be ineffective or even harmful. Broad testing in patients with early-stage NSCLC can provide additional information to guide decisions regarding perioperative therapy and anticipate disease relapse, thereby avoiding treatment delays.

Furthermore, in Brazil, the diagnostic process often involves multiple laboratories. Biopsies are typically processed in a hospital-based or private practice laboratory, confirmatory diagnostic IHC is performed in a referral lab, and molecular and predictive biomarker testing is conducted in a separate facility. In such a complex scenario, it is crucial to follow established protocols that maximize tissue yield and minimize turnaround time. Figure 5 illustrates different testing approaches that can be considered and discussed by local practices in collaboration with the MDT team.

There are two main clinical scenarios, described below:

1. Nonmetastatic resectable non-squamous lung cancer: test for EGFR, ALK and PD-L1 and treat accordingly. Decide in MDT if the block will be sent for multipaneled NGS test right away or subsequently.
2. Metastatic adenocarcinoma: Test for EGFR, ALK and PD-L1 simultaneously by single-gene testing (ROS1

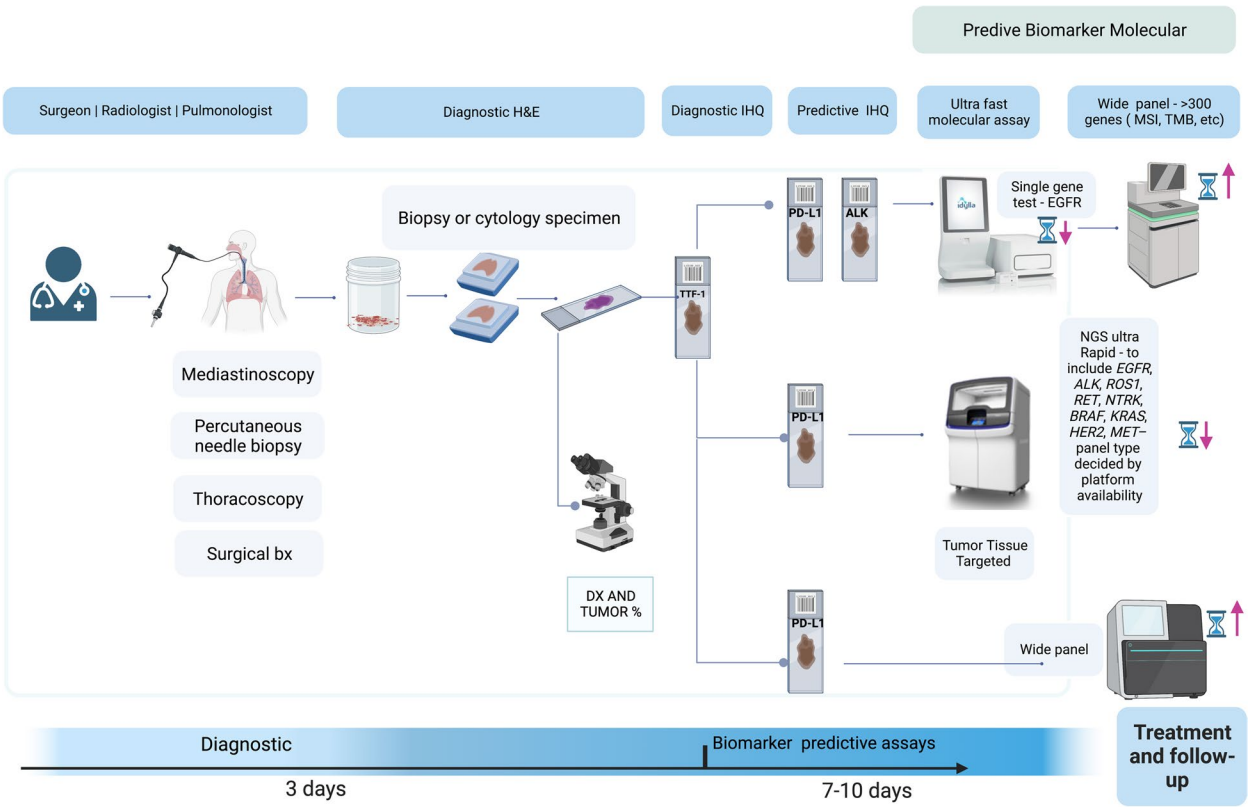

Fig. 5 From diagnostic to biomarker, several decisions should be made by the MDT with participation of the pathologist. In the current scenarios, at least 3 main options are available, based on availability, cost, and possibility of reimbursement

can also be included depending on availability) or test for PD-L1 and perform multigene panel testing for actionable genes.

– *In this second scenario, a single-gene testing can be followed by multipaneled testing if available.*
– *The role of the pathologist is extremely important in consulting with clinicians and decide the best approach for each case.*

The variability in local practices of molecular testing for lung cancer is primarily influenced by the availability of targeted therapies and the public or private scenarios, leading to the development of multiple national or regional guidelines. SBP recommends that each pathologist play a leading role in the local MDTs to decide the testing protocols accordingly. While there are inconsistencies in the recommended test targets among countries, guidelines universally recommend testing for EGFR, ALK, and PD-L1, with BRAF, ROS1 and NTRK being included in most guidelines. Guidelines often recommend testing for KRAS, MET, RET, and ERBB2 (HER2) in a multipaneled NGS or posterior moment. The most current international guidelines, the 2023 ESMO guidelines for oncogene-addicted metastatic NSCLC, recommends biomarkers EGFR, ALK, ROS1, BRAF, RET, MET (including exon 14 skipping and amplification), NTRK, ERBB2 (HER2) mutation, EGFR exon 20 insertion mutation, and KRAS G12C.

The implementation of testing depends on the accessibility of laboratory facilities and the arrangements for test reimbursement. In Brazil, at the time of this writing, there is no universal reimbursement for NGS in either public or health insurance scenarios, and tests are performed in an out-of-pocket or sponsored by the pharmaceutical industry.

Discussion and conclusions

The current manuscript delves into the intricacies of tissue collection, management, processing, and analysis in the realm of lung cancer diagnosis and biomarker reporting. While not intended as a comprehensive text about molecular diagnosis, it is an attempt from a working group from the Brazilian Society of Pathologists to guide recommendations in different scenarios within the country. It underscores the evolving landscape of lung cancer diagnosis, wherein pathological assessment extends beyond mere histological classification, encompassing molecular characterization and predictive biomarker profiling. This shift has led to an increasing reliance on small diagnostic samples, such as biopsies and cytology, surpassing materials derived from complete surgical resection.

The management of small samples presents its own challenges, with scant viable neoplastic cells and the potential for underrepresentation of histological and biological tumor heterogeneity. In navigating these challenges, clinical-radiological correlation emerges as a pivotal aspect of precise sample management. Factors such as lesion location, radiological characteristics, patient medical history, risk factors (like smoking or familial predisposition), specifics of the sampling method, and temporal parameters of sample acquisition are of paramount importance.

A key facet highlighted is the essential role of the pathologist in safeguarding the quality and preservation of the collected tissue. Meticulous orchestration of fixation parameters, encompassing duration and volume, is essential for optimal tissue preservation, crucial for downstream analyses including immunohistochemical staining, in situ hybridization, and DNA/RNA sequencing. Customized protocols for tissue processing, considering available capacity, are recommended.

The pathologist has a central role in the MDT and extends the diagnosis and assessment of tissue quality. Stratification of separate core needles or bronchial biopsies into distinct blocks facilitates diagnostic staining and the preservation of tissue for subsequent molecular analysis. The pathologist's role in ensuring that molecular analysis aligns with the diagnosis articulated in the pathology report, selecting sections devoid of significant necrosis or inflammation, and annotating tumor cell percentage is pivotal.

It is also emphasized the intricate interplay between pathology, clinical context, and imaging findings. It underscores that while an H&E stain can often suffice, judicious use of immunohistochemistry and special stains is crucial for refining diagnoses and guiding subsequent molecular analyses.

PD-L1 testing emerges as a cornerstone, gauging immunotherapy responsiveness. The manuscript elucidates various PD-L1 clones for immunohistochemistry, discussing staining patterns and practical considerations. It accentuates the importance of standardized preanalytical conditions for accurate PD-L1 assessment and the essential role of pathologists in sample selection.

Conclusively, the manuscript underscores the vital role of pathologists in navigating the nuances of lung cancer diagnosis and biomarker profiling. It elucidates the evolving terrain, marked by the rise of small diagnostic samples, the imperative of clinical-radiological correlation, judicious fixation, effective sectioning, and astute sample evaluation. This comprehensive orchestration of skills ultimately contributes to optimized patient outcomes.

Acknowledgements
The authors thank the clerical support of the Brazilian Society of Pathology.

Authors' contributions
Fabio Tavora, Igor Silva, Nicolle Gaglionone, Felipe D'Almeida and Emilio Assis conceptualized the data. Fabio Tavora wrote the article. Clarissa Baldotto, William William and Francisco Neto reviewed the data and test.

Funding
No funding was used in writing this manuscript.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
None.

Received: 10 August 2023 Accepted: 3 September 2023
Published online: 10 October 2023

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